Some histological data of bone and teeth in the Rift Eelpout, *Thermarces cerberus* (Zoarcidae)

by

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Abstract. – The rift eelpout, *Thermarces cerberus* (Zoarcidae), is a deep-sea marine fish that inhabits the vicinity of the hydrothermal vents of the East Pacific Rise. Its skeleton is mineralized to a normal degree and is constituted of acellular bone as the majority of perciforms, to which it belongs. *T. cerberus* feeds on amphipod crustaceans and gastropods, and has thin sharp teeth constituted of an orthodentine cone with a thin external apical layer of enameloid. The histological characteristics of the skeleton (jaws and vertebrae) appear normal with seasonal growth marks. There is no evidence that the physicochemical characteristics of the deep-sea hydrothermal environment of this species play a specific role on its bone anatomy and physiology.

Résumé. – Quelques données histologiques sur l'os et les dents de l'anguille des sources, *Thermarces cerberus* (Zoarcidae).

Thermarces cerberus est un poisson marin de profondeur qui vit près des sources hydrothermales du Rift Est-Pacifique. Son squelette, normalement minéralisé, est constitué d'os acellulaire comme la très grande majorité des Perciformes dont il fait partie. *T. cerberus* se nourrit de crustacés amphipodes et de gastéropodes, et a des dents fines et pointues, constituées d'un cône d'orthodentine coiffé d'une fine couche d'émailloïde. Les caractéristiques histologiques du squelette (mâchoires et vertèbres) paraissent normales, et des marques de croissance saisonnières sont visibles. Les caractéristiques physico-chimiques particulières de son biotope hydrothermal profond ne semblent pas influencer l'anatomie et l'histologie osseuse de cette espèce.

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Thermarces cerberus Rosenblatt & Cohen, 1986, is an eelpout (Zoarcidae) that is a conspicuous member of deep-sea hydrothermal vents of the East Pacific Rise (Cohen *et al*., 1985; Geistdoerfer, 1985; Rosenblatt and Cohen, 1986; Biscoito *et al*., 2002). Hence, *T. cerberus* lives among the vestimentiferan worm communities in a hydrogen sulfid-rich habitat. The physical characteristics of this habitat are peculiar: depth 2600-2700 m, temperature 8-12°C (Geistdoerfer, 1985; Voight, 2000; Biscoito *et al*., 2002; Sancho *et al*., 2005), and these physicochemical environmental conditions may have some influence on the biology of *T. cerberus* (Geistdoerfer and Seuront, 1995), and/or the high hydrostatic pressure at these depths, on the strength of the skeleton (Bertin, 1936). *T. cerberus* is a benthic predator that feeds mainly on gastropods (limpets fixed on the vestimentifer tubes) and amphipod crustaceans (Geistdoerfer and Seuront, 1995; Sancho *et al*., 2005).

Some years ago, the osteology of this species was studied confirming its membership to the Zoarcidae family (Arnulf *et al*., 1987). This anatomical study based on a staining procedure (Alizarin Red S-Alcian Blue) also demonstrated that the skeleton "is rather well ossified, even if most of the bones seems to be fragile" (Arnulf *et al*., 1987). Yet, a histological study was compulsory to confirm the possible fragility of the bones owing, for example, to rickets (Bertin, 1936). The aim of the present study is to confirm previous assertion using classical techniques to study the histology of mineralized tissues.

MATERIAL AND METHODS

Material

The specimens were caught by the expedition "Byocarise" (Ifremer; mars 1984) in the East Pacific: 12°48,80 N; 103°96,60 W); depth: –2620 m. Five precaudal (= abdominal) and five caudal vertebrae were sampled as well as the two lower jaws from a specimen, 250 mm TL.

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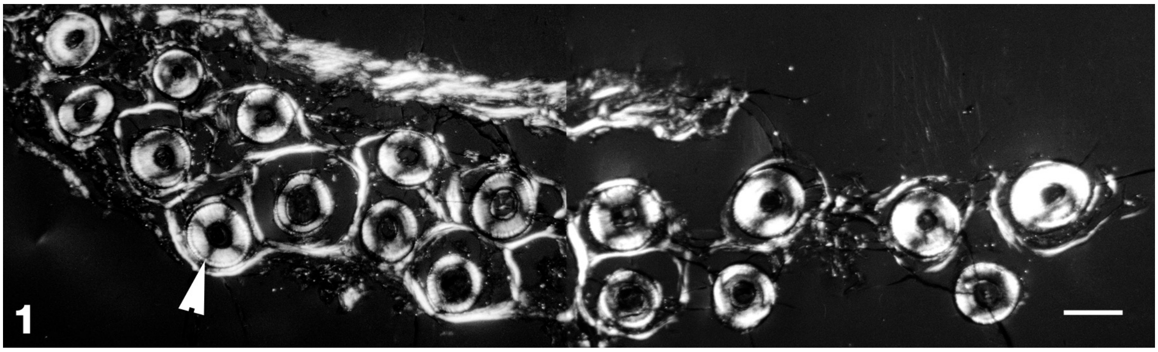
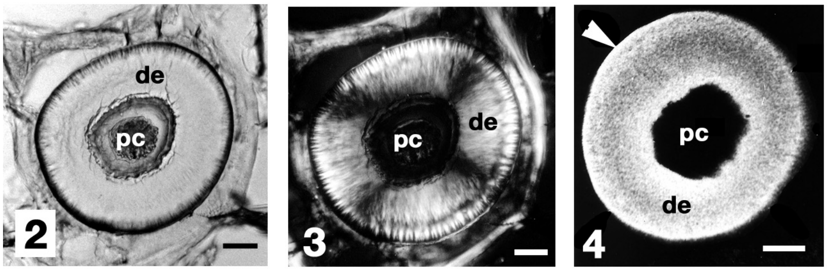
Methods

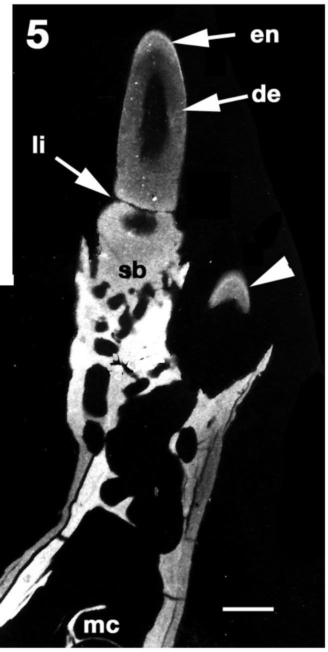
Ground sections. The right lower jaw and the anterior part of the left one were dehydrated in graded series of alcohol till absolute ethanol, transferred to acetone, and embedded in methtyl methacrylate (Juster *et al.*, 1965; Matrajt *et al.*, 1967). Transverse and longitudinal sections (150-200 µm thick) of the right and the left jaws, respectively, were cut with an Isomet sawing machine and ground to 50-75 µm in thickness. The sections were radiographed in a CGR Sigma generator X-ray machine, and then observed under transmitted natural and polarized light with a Zeiss Axiovert 35 microscope.

RESULTS-DISCUSSION

Eelpout teeth form two longitudinal rows on the jaws (Fig. 1). The teeth are sharp, lightly curved, and show a dentine core with a thin external well-mineralized enameloid layer (Figs 2-4). Dentine is crossed by canaliculi that







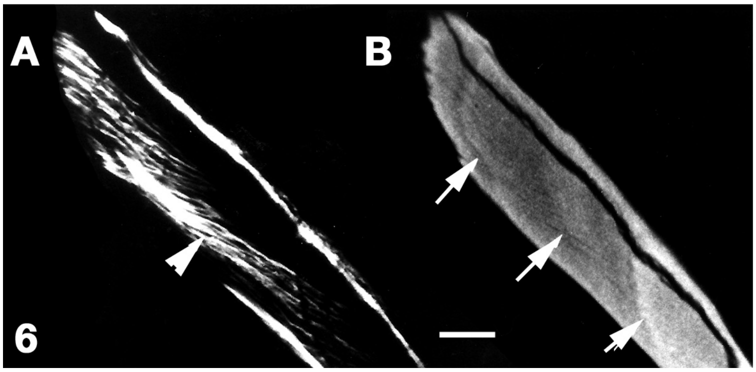


Figure 1. – *Thermarces cerberus*. Horizontal section (transmitted polarized light) of the lower left jaw showing numerous teeth sections aligned on two rows. The arrowhead points to the tooth detailed in figures 2, 3, 4. Scale bar = 500 µm.

Figures 2, 3, 4. – *Thermarces cerberus*. Left lower jaw. Detail of a cross section of a tooth (see arrowhead in Fig. 1), natural transmitted light, polarized light and microradiography, respectively. The arrowhead points to the superficial hypermineralized layer of enameloid (Fig. 4). **De**: dentine; **pc**: pulp cavity. Scale bar = 100 µm.

Figure 5. – *Thermarces cerberus*. Transversal section of the right lower jaw showing an axial section of a tooth. The tooth is fixed on the vascularized supporting bone (**sb**) by an unmineralized ligament (**li**). The dentine core is surrounded by a thin hypermineralized enameloid layer well seen at the tip of the tooth (**en**). At the base of the tooth, on the right, there is a tooth bud (arrowhead) in a lateral alveola of the dentary. **Mc**: Meckel cartilage. Scale bar = 250 µm.

Figure 6. – *Thermarces cerberus*. Detail of a sagittal section of a vertebra. **A**: Polarized light; **B**: Microradiography. Note the fibrillary component of the vertebral bone (arrowhead). The mineralization of the vertebral bone is heterogeneous, and three weakly hypermineralized growth zones are seen (arrows). Scale bar = 100 µm.

are orthogonal to the wall of the pulp cavity and that characterize this tissue as orthodentine. The walls of the pulp cavity are slightly irregular (Fig. 4). Teeth are fixed on the jaw through unmineralized ligaments (Fig. 5) and are thus slightly mobile on their bony support; they belong to the type two of Fink (1981). The bony tissue localized below the tooth is slightly less mineralized than the surrounding jaw bone (Fig. 5). This corresponds to the bone turnover in relation to tooth replacement.

The various bones of *T. cerberus* we have studied look slender and their tissue is totally deprived of osteocytes. There are no osteoblastic processes penetrating the bony tissue either, contrary to certain acellular bony fishes (Sire and Meunier, 2017). The bony tissue of the dentary contains large vascular cavities surrounded by thin bone trabeculae (Fig. 5). Bone remodelling (not illustrated) is observed in some places. The degree of mineralization of the bony tissues appears normal (Figs 5, 6) with the classic heterogeneity (areas of various mineral density) observed in other, more common, studied species like trout, carp and pike (*e.g.* Casselman, 1974; Meunier, 1984; Francillon-Vieillot *et al.*, 1990; Ricqlès *et al.*, 1991; Meunier and François, 1992).

The vertebral bone shows narrow well-mineralized zones and wider, less mineralized, ones (Fig. 6). This alternate pattern characterizes cyclical growth (Meunier, 1988; Castanet *et al.*, 1993). These successive growth marks suggest that the rift eelpout is subjected to a degree of seasonality of its metabolic rate (*e.g.* Meunier and François, 1992; Castanet *et al.*, 1993). This result is rather surprising because the deep-sea environment is generally considered as relatively stable. However, it has been experimentally demonstrated in several species belonging to different vertebrates classes that bone marks are basically related to endogenous rhythms, which can be synchronized and reinforced by environmental cycles (Castanet *et al.*, 1993). Environmental cycles at the hydrothermal vents are unknown, but the reproduction of *T. cerberus* is probably cyclic (Geistdoerfer, 1982), and this breeding cyclicity probably explains the presence of growth marks on the skeleton of the rift eelpout.

CONCLUSION

The presence of acellular bone in *T. cerberus* supports its phylogenetical position within the Zoarcidae among the Perciformes. Bone acellularity is shared by acantopterygian Perciformes with the exception of the Thunninae (*e.g.* Kölliker, 1859; Moss, 1965; Meunier, 1987, 2011; Sire and Meunier, 2017). The skeleton of the rift eelpout shows a normal histological organization for a teleostean fish bearing acellular bone. We have not observed histological features of bone that can be linked to a specific ecological niche in this deep-sea and hydrothermal environment. This confirms the results of the anatomical study by Arnulf *et al.* (1987) that concluded that the flimsy aspect of the skeleton seems to result only of the bones being thin. Our findings do not support the hypothesis that deep marine environments may induce rickets (Bertin, 1936), at least in *T. cerberus*.

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